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Atrial natriuretic factor inhibits metoclopramide stimulated aldosterone release in man

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1 Atrial natriuretic factor (ANF) has an inhibitory effect on angiotensin II and ACTH stimulated aldosterone secretion in man. The selectivity of this aldosterone suppressing effect of ANF is unclear in man. The present study investigated the effect of ANF on the increase in plasma aldosterone due to metoclopramide in man.

2 Eight normal male volunteers were studied on three occasions. Metoclopramide (10 mg slow i.v.) was given on all study days and each volunteer was randomised to receive 45 min infusion of either 5% D-glucose (placebo) or ANF (99–126) 3 or 15 pmol kg^{-1} min^{-1}.

3 Metoclopramide increased plasma aldosterone to approximately 170% of baseline levels ($P < 0.01$). Concomitant infusion of ANF 3 pmol kg^{-1} min^{-1} and 15 pmol kg^{-1} min^{-1} significantly attenuated this rise in plasma aldosterone to approximately 130% ($P < 0.05$) and 110% ($P < 0.01$) of baseline values respectively.

4 It is suggested, in the light of previous findings, that the inhibitory effect of ANF represents a non-selective action of ANF on aldosterone release.

Keywords atrial natriuretic factor metoclopramide aldosterone prolactin adrenocorticotrophin

Introduction

Atrial natriuretic factor (ANF) causes vasorelaxation and natriuresis in humans, actions opposing those of the renin-angiotensin-aldosterone system (RAAS) (Laragh, 1985). There is now a considerable body of evidence to show that ANF inhibits adrenal steroidogenesis in experimental animals. Basal, angiotensin II, K^+, forskolin, PGE_2 and ACTH stimulated aldosterone release are suppressed by ANF in animal studies both *in vitro* and *in vivo* (Atarashi *et al.*, 1985; Chartier *et al.*, 1984; Schiebinger *et al.*, 1988). In man, we and others have previously shown that ANF attenuates the aldosterone response to angiotensin II and ACTH (Anderson *et al.*, 1986; McMurray *et al.*, 1988).

However, as yet, there is still uncertainty on the mechanisms of this aldosterone suppressing effect of ANF and whether ANF suppresses the aldosterone response to all or only some trophic stimuli. A further well known modulator of aldosterone is dopamine (Carey *et al.*, 1979). Metoclopramide, a dopaminergic DA_2-receptor antagonist, is believed to stimulate aldosterone release by causing withdrawal of dopaminergic inhibition of aldosterone release (Edwards *et al.*, 1980; Norbiato *et al.*, 1977; Noth *et al.*, 1980).

We have therefore now conducted a study to investigate the effect of ANF on the aldosterone response to metoclopramide in man. We have also examined the dose-response relationship of ANF with this effect.

Methods

Eight salt replete male volunteers aged 22 to 33 years old (mean 26.5 ± 4.4 years) were studied. All had a normal medical assessment that included physical examination, electrocardiogram (ECG), urinalysis, biochemical profile and haematologic screening. None had taken any regular medication for at least 2 months before the study. Each subject gave written informed consent to the study, which had been approved by the Faculty of Medicine and Dentistry Committee on Medical Ethics.

Subjects were studied on three separate occasions with at least 7 days between each investigational day. Subjects were asked to maintain their usual diet for the duration of the study. Twenty-four hour urine collections

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were made immediately prior to each of these 3 days. This enabled measurement of sodium excretion to ensure maintenance of the sodium balance during the study period.

Each volunteer was asked to attend the clinical laboratory at 14.00 h having fasted from 09.00 h on the morning of each study. Intravenous cannulae were placed in each forearm and subjects remained in the supine position for 120 min. After 45 min, an infusion was commenced which consisted of either a) human ANF (99–126) (Bissendorf Peptides, Wedemark, FRG) 3 pmol kg^{-1} min^{-1} in 5% D-glucose, b) ANF 15 pmol kg^{-1} min^{-1} in 5% D-glucose or c) 5% D-glucose alone (placebo). One of these infusions was administered on each study day in a randomised single-blind fashion and they were all continued for 45 min. Fifteen minutes into the infusion, 10 mg of metoclopramide (Maxalon, Beecham Research Laboratories, Brentford, U.K.) was administered on all 3 study days by slow intravenous injection over 1 min. Subjects remained supine for a further 30 min after the end of the infusion.

Venous blood was taken immediately before infusion of either ANF or placebo, immediately before injection of metoclopramide and at 10, 20 and 30 min after the metoclopramide infusion. A final sample was taken at 30 min after the end of the ANF infusion.

Throughout the study, heart rate was displayed continuously on an ECG oscilloscope and the blood pressure was measured every 10 min with a semi automatic sphygmomanometer (Dinamap Vital Signs Monitor 1946, Critikon, Tampa, Florida).

Venous blood was taken in 10 ml chilled tubes containing lithium heparin for subsequent measurement of aldosterone and cortisol levels. Venous blood was also collected in 10 ml chilled tubes containing 60 mg potassium ethylene diaminetetra-acetic acid (EDTA) and 4000 kallikrein inhibitory units of aprotonin (Trasylol, Bayer U.K., Newbury, Berkshire, U.K.) to measure ANF and ACTH. These tubes were centrifuged at 4° C and the plasma separated and stored at −20° C until assayed. Blood (5 ml) was also taken into plain glass tubes, allowed to clot, and the serum separated and stored at −20° C for later measurement of electrolytes and prolactin concentrations. All assays were performed within 2 months of specimen collection.

Plasma aldosterone and cortisol were measured by commercially available radioimmunoassay kits (Biotex Laboratories Inc., Friendswood, Texas, USA and Immunodiagnostics Ltd, Washington, Tyne and Wear, UK respectively). For the aldosterone assay the inter-assay coefficient of variation (ITCV) was 10% and the intra-assay coefficient (INCV) 1.6%. The equivalent values for the cortisol assay were 4.2% and 2.9%. ANF was measured by radioimmunoassay (Amersham International Ltd, Little Chalfont, Buckinghamshire, U.K.) after plasma extraction. The lower limit of detection of this assay was 4 pmol l^{-1}; ITCV was 12.9% and INCV was 4.6%. Serum prolactin and plasma ACTH were measured by radioimmunoassay using commercially available kits (Chelsea Prolactin Kit, Hammersmith Hospital, London, UK and Diagnostic Products Corporation, Los Angeles, USA respectively). The ACTH assay which was a double antibody radioimmunoassay has an INCV of 5% and an ITCV of 10%. The equivalent values for the prolactin assay were 7% and 12% respectively. Serum electrolytes were measured with an internal caesium standard flame emission photometer (Model 943, Instrumental Laboratory, Milan, Italy).

The statistical significance of the aldosterone and cortisol response to infusion of ANF on each of the experiment days and between the three experimental days was determined by repeated measured analysis of variance (MANOVA, SPSS-Plus) (Nie *et al.*, 1975). This analysis examined the effect of time, treatment (metoclopramide + placebo, metoclopramide + ANF 3 pmol kg^{-1} min^{-1}, metoclopramide + ANF 15 pmol kg^{-1} min^{-1}) and treatment over time. Haemodynamic variables (HR, systolic BP and diastolic BP) were similarly analysed.

Plasma ANF concentrations were compared with baseline (before commencement of infusion) values by paired *t*-test. Plasma ACTH, serum prolactin and electrolytes were similarly evaluated in comparison with baseline (immediately before intravenous 10 mg metoclopramide).

Results

Twenty-four hour urinary sodium excretion (mean ± s.e. mean) on the metoclopramide + P day, metoclopramide + ANF 3 pmol (kg^{-1} min^{-1}) day and metoclopramide + ANF 15 pmol kg^{-1} min^{-1} day were 164 ± 15 mmol, 151 ± 10 mmol and 153 ± 8 mmol respectively which indicated our volunteers were comparably salt replete on all days. There was no significant difference in 24 h urinary sodium excretion in all 3 days. Baseline plasma aldosterone was 416 ± 69 pmol l^{-1} on the metoclopramide + P day, 388 ± 47 pmol l^{-1} on the metoclopramide + ANF 3 pmol kg^{-1} min^{-1} day and 413 ± 49 pmol l^{-1} on the metoclopramide + ANF 15 pmol kg^{-1} min^{-1} study day (no significant difference). Baseline blood pressure, serum electrolytes and prolactin and plasma cortisol and ACTH concentrations were also similar on each study day (Table 1).

Metoclopramide by itself caused a significant increment in plasma aldosterone ($P < 0.01$). Concomitant infusion of ANF 3 pmol kg^{-1} min^{-1} and 15 pmol kg^{-1} min^{-1} significantly attenuated the increase in plasma aldosterone after metoclopramide ($P < 0.05$ and $P < 0.01$ respectively), (Figures 1 and 2).

There was a sharp rise in serum prolactin ($P < 0.01$) and a slight but significant fall in serum potassium ($P < 0.05$) after metoclopramide. These changes were not affected by concomitant infusions of ANF (Table 1).

There was a slight but not significant rise in plasma cortisol after metoclopramide. Plasma ACTH was not affected by metoclopramide. Concomitant infusion of ANF at both doses did not affect these parameters (Table 1).

ANF concentrations did not differ at baseline between study days (Table 1). ANF infusions resulted in significant plasma increments, though the circulating levels achieved differed by a factor of three between the two doses.

Blood pressure and heart rate increased with metoclopramide during each of the three study days, peaking

Table 1 Serum electrolytes, prolactin, cortisol, ACTH and atrial natriuretic factor levels on each of the three study days

	−15 min	*0 min*	*+10 min*	*+20 min*	*+30 min*	*+60 min*
Placebo + metoclopramide						
Na^+ (mmol l^{-1})	–	141.1 ± 0.5	–	–	141.5 ± 0.3	–
K^+ (mmol l^{-1})	–	4.0 ± 0.3	–	–	3.8 ± 0.3*	–
prolactin (m iu l^{-1})	–	138 ± 32	–	–	1581 ± 178**	–
cortisol (nmol l^{-1})	–	244 ± 50	235 ± 47	246 ± 43	302 ± 49	282 ± 42
ACTH (pmol l^{-1})	–	19 ± 10	–	–	14 ± 8	–
ANF (pmol l^{-1})	9.6 ± 2.1	9.2 ± 2.1	–	–	9.7 ± 1.8	–
ANF 3 pmol kg^{-1} min^{-1} + metoclopramide						
Na^+ (mmol l^{-1})	–	141 ± 0.5	–	–	141.2 ± 0.4	–
K^+ (mmol l^{-1})	–	4.1 ± 0.3	–	–	3.8 ± 0.3*	–
prolactin (m iu l^{-1})	–	178 ± 18	–	–	1550 ± 179**	–
cortisol (nmol l^{-1})	–	207 ± 25	200 ± 25	241 ± 44	291 ± 53	251 ± 50
ACTH (pmol l^{-1})	–	18 ± 11	–	–	19 ± 8	–
ANF (pmol l^{-1})	8.1 ± 1.1	43.8 ± 3.2**	–	–	59.3 ± 6.6**	–
ANF 15 pmol kg^{-1} min^{-1} + metoclopramide						
Na^+ (mmol l^{-1})	–	140.5 ± 0.6	–	–	141.2 ± 0.7	–
K^+ (mmol l^{-1})	–	4.1 ± 0.3	–	–	3.8 ± 0.2*	–
prolactin (m iu l^{-1})	–	131 ± 18	–	–	1602 ± 179**	–
cortisol (nmol l^{-1})	–	226 ± 30	193 ± 25	241 ± 44	242 ± 50	227 ± 55
ACTH (pmol l^{-1})	–	13 ± 8	–	–	15 ± 9	–
ANF (pmol l^{-1})	8.8 ± 1.4	145.9 ± 14.3**	–	–	164.8 ± 8.5**	–

*$P < 0.05$; **$P < 0.01$

Values are mean ± s.e. mean. Serum potassium (K^+) decreased with metoclopramide on all study days. These changes were not influenced by atrial natriuretic factor (ANF). There were no significant changes in serum sodium (Na^+) between days or during any study day. Serum prolactin increased with metoclopramide on all study days. These changes were not influenced by ANF. Plasma ANF concentrations increased during ANF infusion. Samples were taken at −15 min (before infusion of placebo or ANF 3 or 5 pmol kg^{-1} min^{-1}), immediately before (0 min) and at +10 min, +20 min, +30 min, +60 min after 10 mg of intravenous metoclopramide.

at +20 min after the intravenous injection of 10 mg metoclopramide (*metoclopramide + P day*: heart rate increased from 59 ± 4 to 68 ± 4 beats min^{-1}**, systolic blood pressure increased from 124 ± 5 to 130 ± 4 mm Hg**, diastolic blood pressure increased from 59 ± 4 to 68 ± 4 mm Hg**; *metoclopramide + ANF 3 pmol kg^{-1} min^{-1} day*: heart rate increased from 58 ± 4 to 68 ± 4 beats min^{-1}**, systolic blood pressure increased from 119 ± 4 to 127 ± 3 mm Hg**, diastolic blood pressure increased from 65 ± 4 to 72 ± 3 mm Hg**; *metoclopramide + ANF 15 pmol kg^{-1} min^{-1} day*: heart rate increased from 63 ± 2 to 71 ± 3 beats min^{-1}**, systolic blood pressure increased from 122 ± 5 to 130 ± 5 mm Hg**, diastolic blood pressure increased from 65 ± 3 to 71 ± 4 mm Hg**; **$P < 0.01$). These changes were however not influenced by ANF at any given time point.

Discussion

In this study, we have shown that ANF inhibits the aldosterone response to metoclopramide in man in an apparent dose-dependent manner. The lower dose infusion of ANF was chosen to achieve a circulating increment within the physiologic range. However, the actual values obtained were higher than anticipated for this dose, reaching about one and a half times the upper limit of normal by the end of the infusion. The levels achieved during the higher dose infusion were comparable, however, to those in patients with chronic heart failure (Shenker *et al.*, 1985).

Previous animal work investigating the effects of ANF on the dopaminergic control of aldosterone secretion have produced conflicting results. Similar attenuation by ANF on the aldosterone release to metoclopramide has been demonstrated in human isolated aldosteronoma cells (Glaz *et al.*, 1988). However, Jungman and co-workers (1986) failed to demonstrate ANF induced inhibition of the aldosterone response to haloperidol in nude mice bearing heterotransplanted human Conn's adenoma tissue. There have been no previous published studies in normal man and thus our study represents the first such study in man.

Metoclopramide, a potent dopaminergic DA_2-receptor antagonist has been consistently shown to increase plasma aldosterone in a variety of species including man (Carey *et al.*, 1979; Edwards *et al.*, 1980). Experiments with other dopamine antagonists have however produced variable results (Aguilera *et al.*, 1984) which was the reason why we chose metoclopramide as our aldosterone secretagogue in this study. The mechanism of metoclopramide's action on aldosterone release is however unclear. It has been suggested that it acts by withdrawing the tonic dopaminergic inhibition on the zona glomerulosa cells. However, metoclopramide has other pharmacological properties aside from dopamine

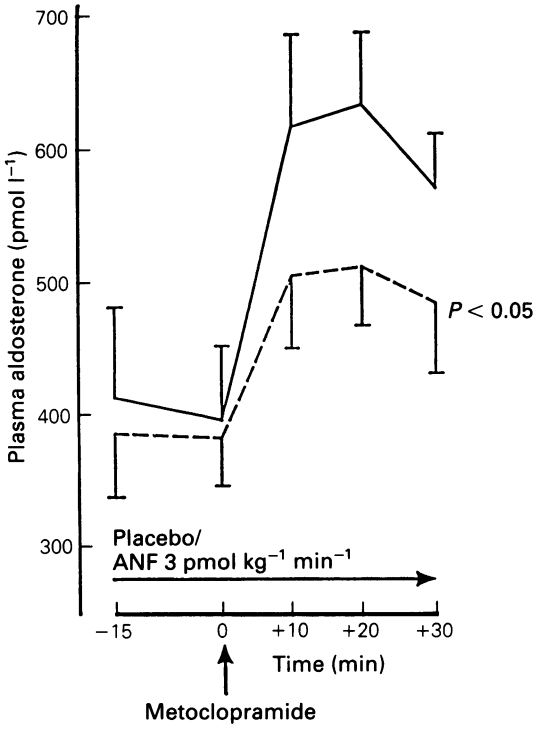

Figure 1 Plasma aldosterone (pmol l^{-1}) mean ± s.e. mean on the placebo plus metoclopramide 10 mg study day (placebo, —) and atrial natriuretic factor 3 pmol kg^{-1} min^{-1} plus metoclopramide 10 mg study day (ANF 3 pmol kg^{-1} min^{-1}, ---) ($n = 8$). The horizontal arrows at the base of the figure indicate the nature and timing of the infusion. The first two points are baseline values. The abscissa shows time from the intravenous injection of metoclopramide 10 mg with the start of the placebo or ANF 3 pmol (kg^{-1} min^{-1}) infusion at time −15 min. ANF 3 pmol kg^{-1} min^{-1} significantly attenuated the rise in plasma aldosterone with metoclopramide ($P < 0.05$ after MANOVA).

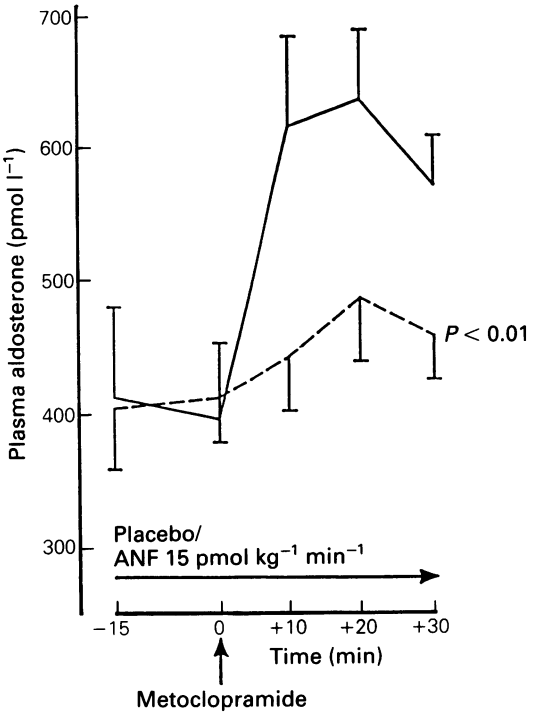

Figure 2 Plasma aldosterone (pmol l^{-1}) mean ± s.e. mean on the placebo plus metoclopramide 10 mg study day (placebo, —) and atrial natriuretic factor 15 pmol kg^{-1} min^{-1} plus metoclopramide 10 mg study day (ANF 15 pmol kg^{-1} min^{-1}, ---) ($n = 8$). The horizontal arrows at the base of the figure indicate the nature and timing of the infusion. The first two points are baseline values. The abscissa shows time from the intravenous injection of metoclopramide 10 mg with the start of the placebo or ANF 15 pmol kg^{-1} min^{-1} infusion at time −15 min. ANF 15 pmol kg^{-1} min^{-1} significantly attenuated the rise in plasma aldosterone with metoclopramide ($P < 0.01$ after MANOVA).

antagonism (Harrington *et al.*, 1983). In fact it has been suggested that the rise in aldosterone may be mediated by metoclopramide's cholinergic properties (De Sommers *et al.*, 1988). Another postulated mechanism is that metoclopramide could cause a transcellular shift of potassium in the adrenal glomerulosa cells which then causes aldosterone release (Bevilacqua *et al.*, 1980). It is therefore of interest in this regard that we found a significant fall in serum potassium after metoclopramide. Overall, however, the available experimental evidence is generally supportive of metoclopramide acting on aldosterone release mainly via dopaminergic effects.

The mechanisms involved in ANF's inhibitory effect on aldosterone biosynthesis and secretion remains unclear. ANF appears to bind to two distinct cell surface receptors on the zona glomerulosa and inhibit the secretion of aldosterone when stimulated by a variety of agonists (Atarashi *et al.*, 1985; Takayanagi *et al.*, 1987). We and others have shown similar inhibition of aldosterone release in man, whether the trophic stimuli was angiotensin II or ACTH (Anderson *et al.*, 1986; McMurray *et al.*, 1988). Our current findings with metoclopramide, yet another aldosterone secretagogue, suggest that this inhibitory effect of ANF is pharmacologically non-selective in man.

ANF receptors are coupled to at least two transducing enzymes, adenylate cyclase and guanylate cyclase (Anand-Srivastava *et al.*, 1985; Tremblay *et al.*, 1985). The exact role of these enzymes remains to be determined although in a recent study, both a rise in cGMP as well as a reduction in cellular cAMP were required for the inhibition of steroidogenesis by ANF (Barret & Isales, 1988). ANF could potentially affect one of several different intracellular second messengers such as calcium, activated kinases and phosphoproteins. Furthermore, whilst some workers have suggested that ANF acts at a 'late' stage in aldosterone biosynthesis i.e. inhibition of the activation of corticosterone methyloxidate (Schiebinger *et al.*, 1988), others have suggested an effect on the 'early' pathway i.e. the step prior to the mitochondrial metabolism of cholesterol (Elliott & Goodriend, 1986). Obviously, any evidence of an effect via an intracellular mediator and/or the aldosterone synthetic pathway in man must remain speculative and cannot be inferred from this study.

The increase in plasma cortisol with 10 mg of metoclopramide was small and occurred only at the 30 min point in the present experiment. Previous investigators, like us, have only evaluated the significance of changes in cortisol concentration between the basal and the response level after administration of metoclopramide and have found no significant difference (Brown *et al.*,

1981; Norbiato *et al.*, 1977). However, when the normal diurnal variation in cortisol was taken into account, a rise in cortisol at 40 min was observed after administration of metoclopramide (Nishida *et al.*, 1983a). The mechanism of this rise in cortisol remains unclear although it could perhaps be due to metoclopramide causing stress and hence ACTH secretion (Nishida *et al.*, 1983b). This could have been true in the present study since the volunteers almost always developed transient restlessness (without any detectable increase in extrapyramidal activity) lasting 10–30 min after administration of metoclopramide which was accompanied by a rise in both heart rate and blood pressure. The absence of a detectable rise in ACTH concentration may however, be due to the shorter circulating half life of ACTH, 3–8 min as opposed to approximately 70 min for cortisol (Krieger *et al.*, 1979). Nevertheless our finding of a lack of effect of ANF on cortisol secretion is in good agreement with animal *in vivo* and *in vitro* studies. High affinity receptors for ANF have been identified on glomerulosa cells but few, if any, are found in the adrenal fasiculata (Bianchi *et al.*, 1985). Thus it would appear that ANF acts only on cells involved in aldosterone but not the glucocorticorticoid synthetic pathways.

Our other finding of a lack of effect on prolactin secretion may simply be attributed to the inability of peripherally administered exogenous ANF to cross the blood-brain barrier to act centrally in influencing prolactin secretion. There is evidence from animal studies to show that whilst ANF does not alter basal or stimulated prolactin release in dispersed anterior pituitary cells, ANF when administered intracerebrally, will suppress prolactin secretion in conscious orchidectomized rats (Samson *et al.*, 1988; Samson & Bianchi, 1988). Furthermore, domperidone reverses this inhibitory effect thus indicating that ANF's inhibitory action on prolactin release is in part via an interaction with endogenous dopaminergic systems and is within the hypothalamus.

In summary, this study shows that in man, ANF attenuates in an apparent dose dependent manner the plasma aldosterone response but not the prolactin nor cortisol response to metoclopramide. Taken in conjunction with previous findings these observations further suggest that ANF inhibits aldosterone biosynthesis in man in a non-selective manner.

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